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(21) International Application Number: <b>PCT/US99/07038</b> (22) International Filing Date: 13 May 1999 (13.05.99)  (30) Priority Data: 09/081,164 18 May 1998 (18.05.98) US  (71) Applicant ( <i>for all designated States except US</i> ): PHARMACIA & UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US).  (72) Inventor; and (75) Inventor/Applicant ( <i>for US only</i> ): BOHANON, Michael, John [US/US]; 31640 28th Avenue, Gobles, MI 49055 (US).  (74) Agent: YANG, Lucy, X.; Intellectual Property Legal Services, Pharmacia & Upjohn Company, 301 Henrietta Street, Kalamazoo, MI 49001 (US).	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
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**(54) Title:** ENHANCEMENT OF OXAZOLIDINONE ANTIBACTERIAL AGENTS ACTIVITY BY USING ARGinine DERIVATIVES**(57) Abstract**

The present invention provides methods and compositions for enhancing the effectiveness of oxazolidinone antibacterial agents against gram-negative organisms infection by using an arginine derivative.

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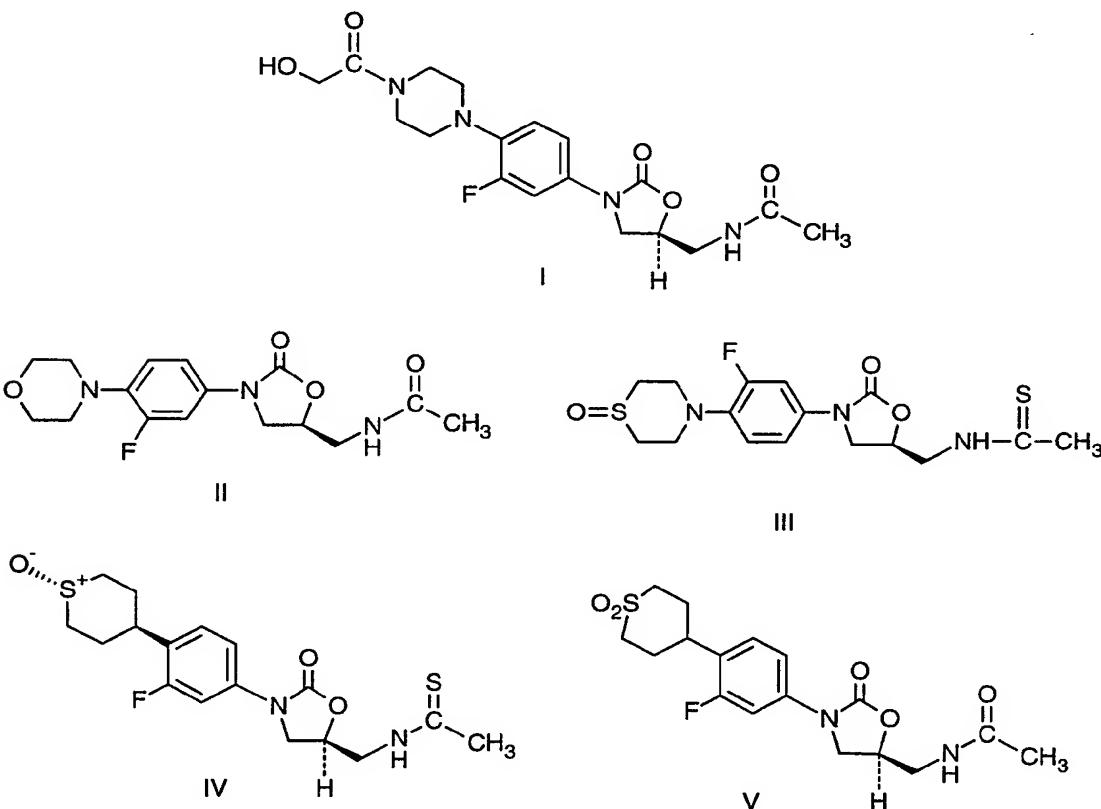
ENHANCEMENT OF OXAZOLIDINONE ANTIBACTERIAL AGENTS  
ACTIVITY BY USING ARGININE DERIVATIVES

FIELD OF THE INVENTION

5        The present invention relates to methods and compositions for enhancing the effectiveness of oxazolidinone antibacterial agents against gram-negative organisms by using an arginine derivative.

BACKGROUND OF THE INVENTION

10       The oxazolidinone antibacterial agents are a novel synthetic class of antimicrobials with potent activity against a number of human and veterinary pathogens, including gram-positive aerobic bacteria such as multiply-resistant staphylococci and streptococci, anaerobic organisms such as bacteroides and clostridia species, and acid-fast organisms such as *Mycobacterium tuberculosis* and *Mycobacterium avium*. In particular, the  
15       oxazolidinone compounds of structures I - V have been found especially effective.



However, certain oxazolidinones generally have poor activity at a useful level against aerobic gram-negative organisms such as *E. coli*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, or *Klebsiella pneumoniae*. Thus, the use of these oxazolidinone antibacterial agents alone is limited to infectious states due to gram-positive bacteria. Accordingly, it is among the objects of the present invention to provide a process for enhancing the inhibitory spectrum of the oxazolidinones. We have now discovered that when the oxazolidinone antibacterial agents are administered with an arginine derivative, a pronounced synergic effect against gram-negative organisms is produced. The effective amount of an oxazolidinone antibacterial agent to be fully efficacious against aerobic gram-negative organisms is much lower than would be needed if an oxazolidinone was administered without these arginine derivatives.

#### INFORMATION DISCLOSURE

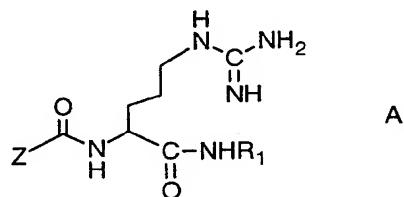
International publication No. WO 96/33285 discloses methods for screening inhibitors of microbial efflux pumps, including those which export antibiotics. The screening methods are based on the increase in the intracellular concentration of a compound, such as an antibiotic or a dye, when the bacterial cells are contacted with an efflux pump inhibitor. In addition, this invention provides pharmaceutical compositions containing such efflux pump inhibitors, including certain arginine derivatives, and methods for treating microbial infections and enhancing the antimicrobial activity of certain antimicrobial agents.

The abstract of the 36th ICAAC, presented by Pharmacia and Upjohn, Inc. discloses mutation of the AcrAB antibiotic efflux pump in *E. coli* confers susceptibility to oxazolidinone antibacterial agents.

25

#### SUMMARY OF THE INVENTION

The present invention presents a method and a composition for treating gram-negative organisms infections in mammal which comprises administration of an effective amount of an oxazolidinone antibacterial agent and an arginine derivative of formula A



30

wherein

R<sub>1</sub> is

- 5        a)      aryl, optionally substituted with C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> alkythio, halo, or -NH<sub>2</sub>,
- b)      -(CH<sub>2</sub>)<sub>i</sub>-aryl, in which aryl is substituted with C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> alkythio, halo, or -NH<sub>2</sub>,
- c)      thienyl, furyl, pyridyl, benzofuranyl, or benzothienyl;

Z is R<sub>2</sub>, or -CHWR<sub>2</sub>;

R<sub>2</sub> is

- 10      a)      aryl, optionally substituted with one or two C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> alkythio, halo, -NH<sub>2</sub>, C<sub>1-4</sub> alkylamino, C<sub>1-4</sub> dialkylamino, or -NHOH,
- b)      C<sub>1-4</sub> alkyl, optionally substituted with fluoro,
- c)      C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> alkythio,
- d)      halo,
- 15      e)      thienyl, furanyl, or pyridyl;

W is H, -NH<sub>2</sub>, C<sub>1-4</sub> alkylamino, C<sub>1-4</sub> dialkylamino, halo, hydroxyl, C<sub>1-4</sub> alkoxy, alkylthio, or azaheterocycle;

aryl is phenyl, or naphthyl;

azaheterocycle is n-morpholinyl, n-piperazinyl, n-pyrrolidinyl, n-imidazolyl, n-pyrrolyl, n-pyrazolyl, n-triazolyl, or n-tetrazolyl;

i is 0, 1, or 2; and pharmaceutically acceptable salts.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention teaches that when oxazolidinone antibacterial agents are administered with an arginine derivative, the oxazolidinones are effective against aerobic gram-negative organisms such as *E. coli*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, or *Klebsiella pneumoniae*, as well as gram-positive aerobic bacteria such as multiply-resistant staphylococci and streptococci, anaerobic organisms such as bacteroides and clostridia species, and acid-fast organisms such as *Mycobacterium tuberculosis* and *Mycobacterium avium*. The effective amount of an oxazolidinone antibacterial agent to be fully efficacious against aerobic gram-negative organisms is much lower than would be needed if an oxazolidinone was administered without these arginine derivatives.

For the purpose of the present invention, the term "C<sub>1-4</sub> alkyl" refers to an alkyl group having one to four carbon atoms, such as, methyl, ethyl, propyl, butyl and their isomeric forms thereof.

The term "C<sub>1-4</sub> alkoxy" refers to an alkyl group having one to four carbon atoms attached to an oxygen atom of hydroxyl group, such as, methoxy, ethoxy, propyloxy, butyloxy and their isomeric forms thereof.

The term "C<sub>1-4</sub> alkythio" refers to an alkyl group having one to four carbon atoms and isomeric forms thereof attached to a sulfur atom.

The term "C<sub>1-4</sub> alkylamino" refers to an alkyl group having one to four carbon atoms attached to an amino moiety; for example, methylamino, ethylamino, n-propylamino, n-butylamino and isomeric forms thereof.

The term "C<sub>1-4</sub> dialkylamino" refers to two alkyl groups having one to four carbon atoms attached to an amino moiety; for example, dimethylamino, methylethylamino, diethylamino, dipropylamino, methypropylamino, ethylpropylamino, dibutylamino and isomeric forms thereof.

The term halo refers to fluoro, chloro, bromo, or iodo, preferably fluoro, chloro, or bromo.

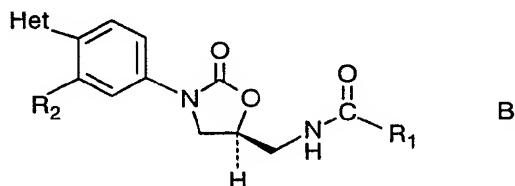
The term "aryl" refers to phenyl or naphthyl;

The term "azaheterocycle" refers to n-morpholinyl, n-piperazinyl, n-pyrrolidinyl, n-imidazolyl, n-pyrrolyl, n-pyrazolyl, n-triazolyl, or n-tetrazolyl;

The term "pharmaceutically acceptable salts" refers to salts useful for administering the compounds of this invention and include hydrochloride, hydrobromide, hydroiodide, sulfate, phosphate, acetate, propionate, lactate, mesylate, maleate, malate, succinate, tartrate, citrate, 2-hydroxyethyl sulfonate, fumarate and the like. These salts may be in hydrated form.

The term "mammal" refers to man or animals of veterinary interest.

Oxazolidinone antibacterial agents refer to compounds of formula B



wherein R<sub>1</sub> is methyl, ethyl, cyclopropyl, or dichloromethyl; R<sub>2</sub> is hydrogen or fluoro;

Het is a 6-membered saturated heterocyclic moiety having one to two atoms selected from the group consisting of sulfur, nitrogen and oxygen atoms. Optionally, the nitrogen atom of the heterocyclic may be substituted by a suit group such as hydroxyacetyl, and the sulfur atom may be oxidized. In addition, the compound of formula Y embraces all possible 5 stereoisomers and geometric forms. Preferably, the oxazolidinone antibacterial agents are the compounds of formulas I - V as identified above.

There are numerous references in the art disclosing a variety of oxazolidinone derivatives and the methods of making them. The above oxazolidinone antibacterial agents may be made according to the procedures described in US patent Nos. 5,652,238 and 10 5,688,792, International Publications Nos. WO 93/23384, WO 97/09328 and WO 98/54161, incorporated herein by reference.

Arginine derivatives of formula A are known and readily available or may be prepared by synthetic chemistry methods known to those skilled in the arts. Preferably, arginine derivatives of formula A is L-phenylalanyl-Larginyl- $\beta$ -naphthylamide.

15 The pharmaceutical compositions of this invention comprise an oxazolidinone antibacterial agent and an arginine derivative of formula A, together with one or more solid or liquid pharmaceutically acceptable carriers and optionally pharmaceutically acceptable adjuvants and excipients. Solid form compositions include powders, tablets, dispersible granules, capsules and suppositories. A solid carrier can be at least one substance which 20 may also function as a diluent, flavoring agent, solubilizer, lubricant, suspending agent, binder, tablet disintegrating agent, and encapsulating agent. Inert solid carriers include magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, cellulosic materials, low melting wax, cocoa butter, and the like. Liquid form compositions include solutions, suspensions and emulsions. For example, there may be 25 provided solutions of the compounds of this invention dissolved in water, water-propylene glycol, and water-polyethylene glycol systems, optionally containing conventional coloring agents, flavoring agents, stabilizers and thickening agents.

The pharmaceutical compositions are provided by employing conventional techniques. Preferably the compositions are in unit dosage form containing an effective 30 amount of an oxazolidinone antibacterial agent the compounds of formula B.

The amount of an oxazolidinone antibacterial agent in the pharmaceutical composition and unit dosage form thereof may be varied or adjusted widely depending upon the particular application method, the potency of the particular compound, the condition to

be treated and the desired concentration. Generally, the quantity of an oxazolidinone antibacterial agent will range between 0.5% to 90% by weight of the total composition.

In therapeutic use for treating bacterial infections in humans and other animals that have been diagnosed with aerobic gram-negative organisms infections, the oxazolidinone antibacterial agent and arginine derivatives or pharmaceutical compositions of the present invention thereof will be administered orally, parenterally, transdermally and/or topically at a dosage to obtain and maintain a concentration, that is, an amount, or blood-level of active component in the animal undergoing treatment which will be antibacterially effective. The preferred form of administration is orally. Generally, such antibacterially effective amount of dosage of active oxazolidinone antibacterial agent will be in the range of about 0.1 to about 100 mg/kg normal body weight, more preferably about 3.0 to about 50 mg/kg of body weight/day. It is to be understood that the dosages may vary depending upon the requirements of the patient, the severity of the bacterial infection being treated, and the particular compounds being used. Also, it is to be understood that the initial dosage administered may be increased beyond the above upper level in order to rapidly achieve the desired blood-level, or the initial dosage may be smaller than the optimum, and the daily dosage may be progressively increased during the course of treatment depending on the particular situation. If desired, the daily dose may also be divided into multiple doses for administration, e.g., two to four times per day.

The amount of an arginine derivative of formula A to be used varies with the enhancing activity of the particular arginine derivative and its absorption by the organism being treated. Sufficient amounts of the arginine derivatives should be used to make aerobic gram-negative organisms susceptible to a pharmaceutically acceptable level of an oxazolidinone antibacterial agent in mammals being treated. The sufficient amount of a particular arginine derivative can simply be determined by testing for minimum inhibitory concentration (MIC) of an oxazolidinone antibacterial agent, and comparing the MIC of that antibacterial agent alone, with the MIC of that antibacterial agent used in combination with the arginine derivative. Generally, the molar ratio of an arginine derivative to an oxazolidinone antibacterial agent which is administered may be from about 0.01 to 10, preferably from about 0.1 to 1.0. Therefore, the daily dosage of an arginine derivative for enhancing the effectiveness of oxazolidinone antibacterial agents against aerobic gram-negative organisms in mammals may range from about 0.01 to 100 mg/kg normal body weight, preferably in an amount of about 0.3 to 50 mg/kg body weight. The arginine

derivatives may be administered one to four hours before the oxazolidinone antibacterial agents is administered, or is administered simultaneously with the oxazolidinone antibacterial agents.

5

#### BIOLOGICAL TESTING

The potentiation of oxazolidinone antibacterial agents activity against aerobic gram-negative organisms when combined with an arginine derivative of formula A uses two assay techniques:

- 10        a) conventional checkerboard technique, and
- b) silicone-oil centrifugation method for quantitating an radiolabelled oxazolidinone antibacterial agent in *E. coli*.

I. Determination of Fractional Inhibitory Concentration (FIC) Index by Using Checkerboard Technique.

15        The "checkerboard" method is the *in vitro* technique used most often to assess antimicrobial combinations (Lorian, V., editor. *Antibiotics in Laboratory Medicine*, Third Edition, p. 432, Williams & Wilkins. Baltimore, Maryland 21202, USA). In the microdilution method, a checkerboard pattern is formed in the wells of microtiter plates that contains multiple 2-fold dilutions of the two agents being tested. The test dilutions span a range of concentrations that are above and below the Minimum Inhibitory Concentration (MIC) of each test agent for the test organism. The response at each test ratio (growth or no growth) is used for the calculation of the Fractional Inhibitory Concentration (FIC) Index. The drug-drug interaction is defined as Additive when the result for the two drugs together is equal to the sum of the results for each of the two drugs used separately (FIC Index= 1.0).  
20        The interaction is described as Antagonism when the result for the two drugs is significantly less than the Additive response (FIC Index >1.0). The interaction is described as synergistic when the result for the two drugs combined is significantly greater than the Additive response (FIC Index £ 0.50).

30        II        Quantitating a Radiolabelled Oxazolidinone Antibacterial agent of Formula I Accumulation in *E. coli*. Following Pre-Treatment of the Bacterial Cells with an Arginine Derivative of Formula A.

Measurement of radiolabelled oxazolidinone antibacterial agent of formula I accumulation in *E. coli* was conducted according to the procedures described by Thanassi, D.G., G.S.B. Suh, H. Nikaido, J. Bacteriology, 1995, p. 177, (4):998-1007. Briefly, cells were grown to mid log ( $OD_{530}$  0.5-0.7) in LB/0.2% glucose at 37° C, harvested by centrifugation, washed twice and resuspended to an  $OD_{530}$  of 8.0 in 50 mM potassium phosphate pH 7.0, 1 mM MgSO<sub>4</sub> and 0.2% glucose. 1.0 ml aliquots of the cell suspension were preincubated at 37° C for 10 minutes prior to the addition of an arginine derivative. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) was used as the positive control. After addition of an arginine derivative, L-phenylalanyl-Larginyl-β-naphthylamide, the cells were incubated for 30 minutes before the addition of the radiolabelled oxazolidinone antibacterial agent of formula I to a final concentration of 25 TM. The cells were then incubated for an additional 15 minutes. A 50 Tl aliquot was removed and layered on a 300 Tl silicone oil cushion (70% fluid no. 550 and 30% fluid no. 510 silicone oil, Dow Corning Corp. Midland, MI). Tubes were centrifuged at 12,000 rpm for 3 minutes, 22° C, then 15 frozen by emersion into liquid N<sub>2</sub>. The tips of each tube containing the cell pellets were cut off and placed in scintillation vials. After thawing, the cell pellets were suspended in 200 Tl distilled water and 4 ml of scintillation fluid was added. The samples were mixed well and counted in a liquid scintillation counter. To correct for nonspecific adherence of the labelled drug to the cell surface, a control experiment was done with cells incubated 20 throughout with only the addition of vehicle and radiolabelled oxazolidinone antibacterial agent of formula I.

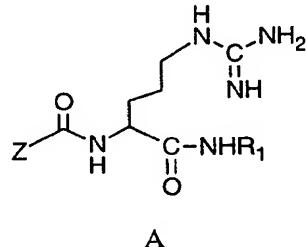
### III. RESULTS

The oxazolidinone antibacterial agent of formula I alone demonstrated very poor 25 antibacterial activity, requiring a concentration of 256 mg/ml to inhibit *E. coli* 31700. L-Phenylalanyl-Larginyl-β-naphthylamide alone also demonstrated very poor antibacterial activity as evidenced by no growth inhibition up to a concentration of 256 mg/ml. However, when the two agents were combined in the "checkerboard" pattern, there was dramatic evidence of the potentiation of the activity of oxazolidinone antibacterial agent of 30 formula I by L-phenylalanyl-Larginyl-β-naphthylamide. For example, in the presence of 16 mg/ml L-phenylalanyl-Larginyl-β-naphthylamide, an oxazolidinone antibacterial agent of formula I concentration of 16 mg/ml was inhibitory for bacterial growth. Calculation of the FIC Index yielded a value of 0.23, clearly indicative of a synergistic interaction.

## CLAIMS

We claim

1. A method for treating gram-negative organisms infections in mammal which comprises administration of an effective amount of an oxazolidinone antibacterial agent and  
5 an arginine derivative of formula A



wherein R<sub>1</sub> is

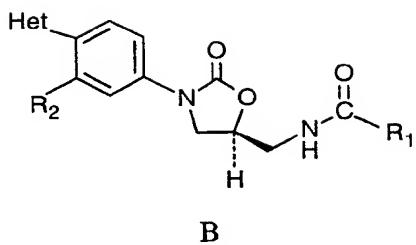
- a) aryl, optionally substituted with C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> alkythio, halo, or -NH<sub>2</sub>,
- 10 b) -(CH<sub>2</sub>)<sub>i</sub>-aryl, in which aryl is substituted with C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> alkythio, halo, or -NH<sub>2</sub>, or
- c) thienyl, furyl, pyridyl, benzofuranyl, or benzothienyl;

Z is R<sub>2</sub>, or -CHWR<sub>2</sub>;

15 R<sub>2</sub> is

- a) aryl, optionally substituted with one or two C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> alkythio, halo, -NH<sub>2</sub>, C<sub>1-4</sub> alkylamino, C<sub>1-4</sub> dialkylamino, or -NHOH,
- b) C<sub>1-4</sub> alkyl, optionally substituted with fluoro,
- c) C<sub>1-4</sub> alkoxy,
- 20 d) C<sub>1-4</sub> alkythio,
- e) halo, or
- f) thienyl, furanyl, or pyridyl;

- W is H, -NH<sub>2</sub>, C<sub>1-4</sub> alkylamino, C<sub>1-4</sub> dialkylamino, halo, hydroxyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> alkylthio, or azaheterocycle;
- 25 aryl is phenyl, or naphthyl;
- azaheterocycle is n-morpholinyl, n-piperazinyl, n-pyrrolidinyl, n-imidazolyl, n-pyrrolyl, n-pyrazolyl, n-triazolyl, or n-tetrazolyl;
- i is 0, 1, or 2; and pharmaceutically acceptable salts.
2. The method of claim 1 wherein the oxazolidinone antibacterial agent is compounds  
30 of structure B

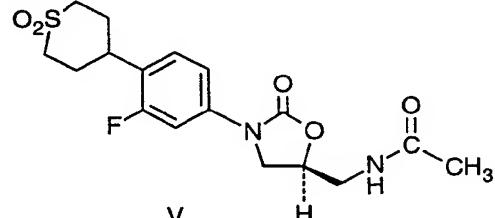
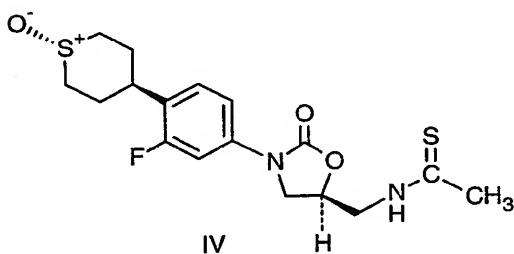
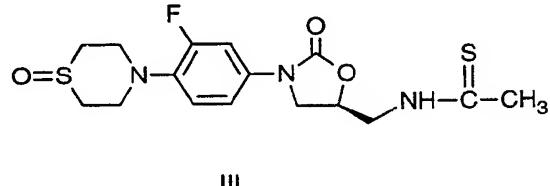
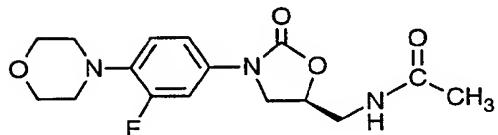
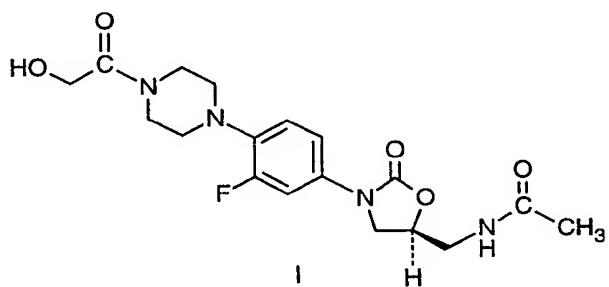


wherein R<sub>1</sub> is methyl, ethyl, cyclopropyl, or dichloromethyl;

R<sub>2</sub> is hydrogen or fluoro; and

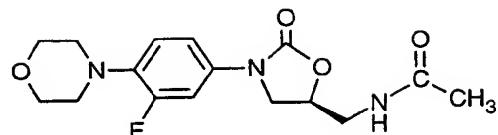
- 5 Het is a 6-membered saturated heterocyclic moiety having one to two atoms selected from the group consisting of sulfur, nitrogen and oxygen atoms.

3. The method of claim 2 wherein the oxazolidinone antibacterial agent is a compound of structures I, II, III, IV or V.



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4. The method of claim 2 wherein the oxazolidinone antibacterial agent is a compound of structure II.



II

5. The method of claim 1 wherein the arginine derivative of formula A is  
5 L-phenylalanyl-Larginyl- $\beta$ -naphthylamide.
6. The method of claim 1 wherein the oxazolidinone antibacterial agent and the  
arginine derivative of formula A are administered in a ratio of 10 (the oxazolidinone  
antibacterial agent) : 0.01(the arginine derivative of formula A).
- 10
7. The method of claim 1 wherein the oxazolidinone antibacterial agent and the  
arginine derivative of formula A are administered in a ratio of 1 : 1.
- 15
8. The method of claim 1 wherein the effective amount of oxazolidinone antibacterial  
agent is from about 0.1 to about 100 mg/kg of body weight/day.
9. The method of claim 1 wherein the effective amount of oxazolidinone antibacterial  
agent is from about 3 to about 50 mg/kg of body weight/day.
- 20
10. The method of claim 1 wherein the amount of the arginine derivative of formula A  
is from about 0.01 to about 100 mg/kg of body weight/day.
11. The method of claim 1 wherein the amount of the arginine derivative of formula A  
is from about 0.3 to about 50 mg/kg of body weight/day.
- 25
12. The method of claim 1 wherein the oxazolidinone antibacterial agent and the  
arginine derivative of formula A are administered simultaneously.
13. The method of claim 1 wherein the arginine derivative of formula A is  
30 administrated about one to four hours before the oxazolidinone antibacterial agent is  
administered.

14. The method of claim 1 wherein the effective amount of an oxazolidinone antibacterial agent and an arginine derivative of formula A are administered orally, parenterally, transdermally or topically.

5

15. The method of claim 1 wherein the gram-negative organisms are aerobic gram-negative organisms.

16. The method of claim 15 wherein the aerobic gram-negative organism is *E. coli*,  
10 *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, or *Klebsiella pneumoniae*.

17. A composition for treating gram-negative organisms infections in mammal which comprises an oxazolidinone antibacterial agent of formula B, an arginine derivative of  
15 formula A and a pharmaceutically acceptable carrier.

18. The composition of claim 17 wherein the oxazolidinone antibacterial agent is compounds of structures I, II, III, IV or V.

20 19. The composition of claim 17 wherein the arginine derivative of formula A is L-phenylalanyl-Larginyl-β-naphthylamide.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/07038

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 6	A61K38/05	A61K31/54	A61K31/535	A61K31/495	A61K31/42
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According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category <sup>o</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 33285 A (MICROCIDE PHARMACEUTICALS INC ;CHAMBERLAND SUZANNE (US); HECKER SC) 24 October 1996 (1996-10-24) cited in the application abstract page 75, line 23 -page 79, line 23 -----	1-19
A,P	WO 99 17791 A (TUFTS COLLEGE) 15 April 1999 (1999-04-15) claims -----	1-19

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

<sup>o</sup> Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

4 October 1999

Date of mailing of the international search report

13/10/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Leherte, C

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US 99/07038

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: 1-16 because they relate to subject matter not required to be searched by this Authority, namely:  
**Remark:** Although claims 1-16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.  Claims Nos.: - because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
See FURTHER INFORMATION SHEET PCT/ISA/210
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest** The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-19 relate to an extremely large number of possible compositions. In fact, the claims contain so many possible combinations that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely those compounds recited in the examples.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 99/07038

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
WO 9633285	A 24-10-1996	AU 5713496 A	CA 2217865 A	EP 0823942 A	07-11-1996 24-10-1996 18-02-1998
				JP 11503913 T	06-04-1999
WO 9917791	A 15-04-1999	AU 6578598 A			27-04-1999